Microwave Probing of Protein Interactions

Kimberly Taylor
Daniel van der Weide
University of Wisconsin-Madison
& vdW Design, Middleton WI USA

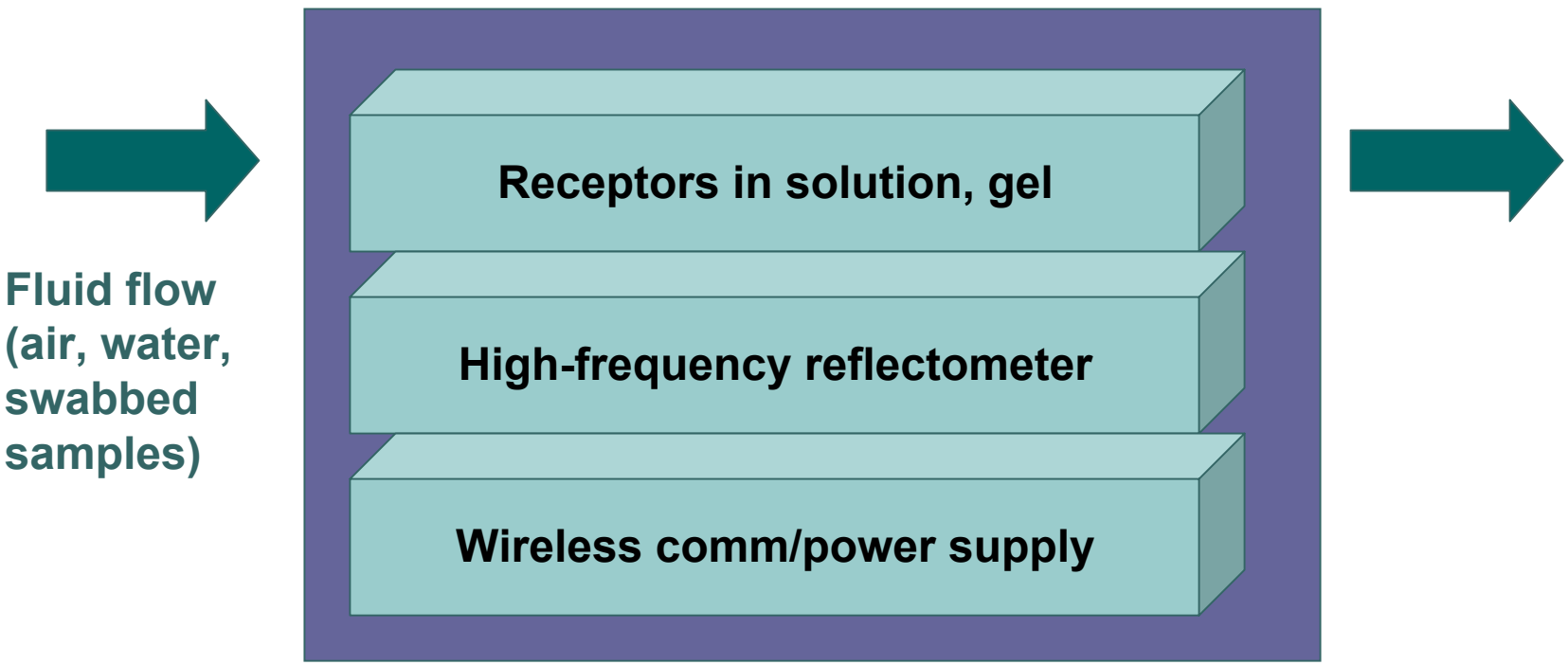
Overview

- Dielectric properties of biological macromolecules
- Slot antenna system
- Experimental results
  - Protein unfolding/refolding thermodynamics
  - Ligand binding
- Conclusions

Objective

- Detection of changes in conformation of biological macromolecules in solution is central to biodetection for security
  - Folding/unfolding (protein)
  - Association, hydridization
  - Ligand binding
  - Channel/pore activity
- Applications for ultrasensitive detection
  - Monitoring water supplies
  - Monitoring air quality
  - Monitoring surfaces, package contents for toxins

Field-deployable ultrasensitive biodetection is now possible in chip format



Conventional methods of detection are optical, thermal or mechanical

- Spectroscopic
  - UV/VIS, circular dichroism
  - Fluorescence, NMR
- Calorimetric
  - Different scanning, isothermal titration
- Other
  - Analytical ultracentrifugation
  - Electrophoresis
  - Surface plasmon resonance (SPR)

Dielectric dispersion enables ultrasensitive electrical detection

- Permittivity (ε): measure of polarization of a material

$$\mathbf{P} = \left[\varepsilon(\omega) - 1\right]\varepsilon_0 \mathbf{E}$$

$$\varepsilon(\omega) = \varepsilon'(\omega) - j\varepsilon''(\omega)$$

Dielectric dispersion

- At low frequency, dipoles attempt to rotate with external field
- At higher frequency, dipoles can no longer keep pace with field
- Resonant frequency: frequency at which ε″ reaches a local maximum
- For proteins, f_r is proportional to size

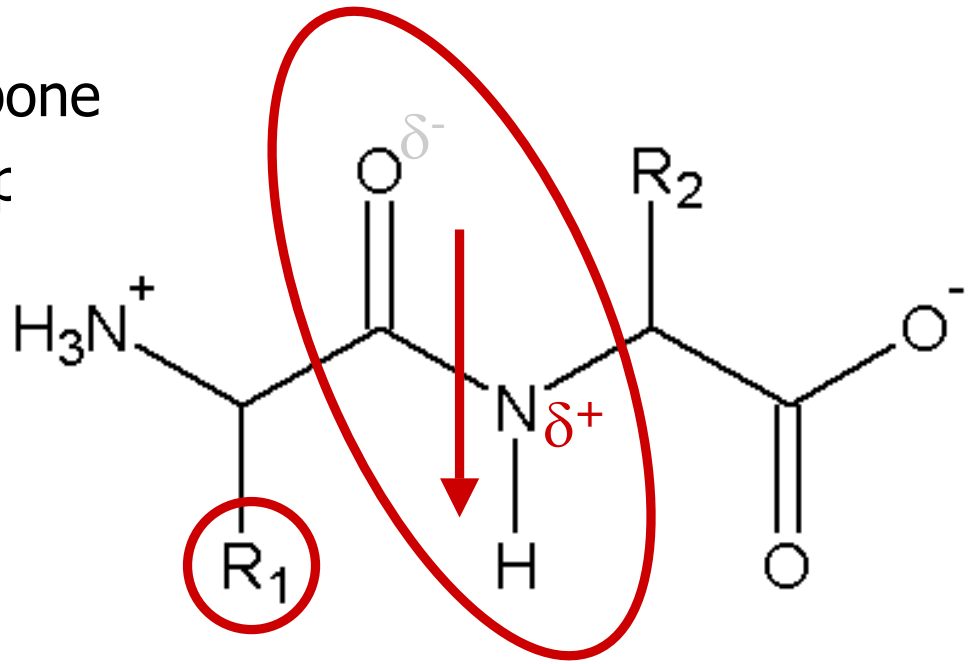
$$f_r = \frac{\omega_r}{2\pi} = \frac{kT}{8\pi^2 \eta r^3}$$

Dipole sources in biological macromolecules

- Protein: backbone and charged or polar residues
- DNA/RNA: sugar, phosphate groups, associated charges
- Lipids: charged or polar head group; interfacial effects with hydrophobic tails
- Dispersion from these macromolecules enhanced by presence of water

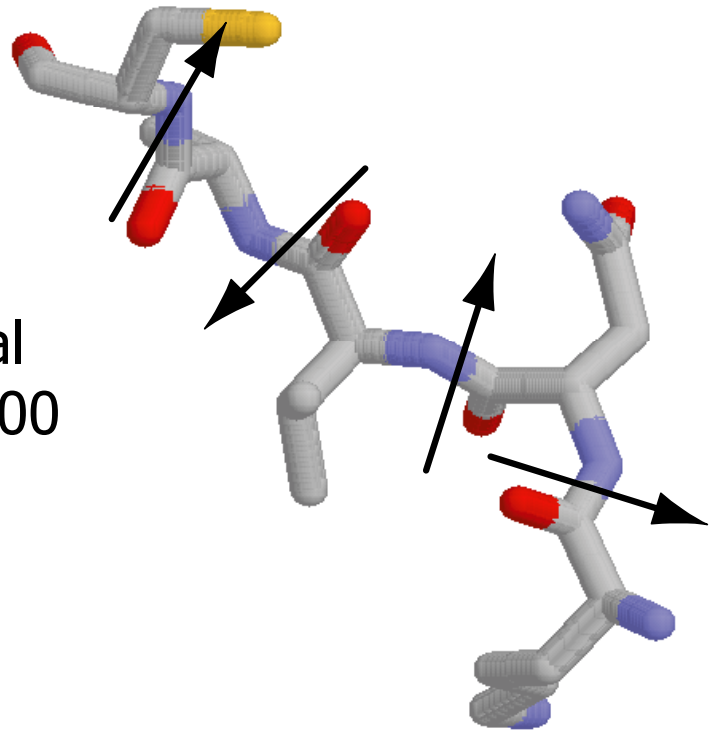
Dielectric response of proteins

- Dipole sources
  - Peptide backbone
  - Charged and p residues

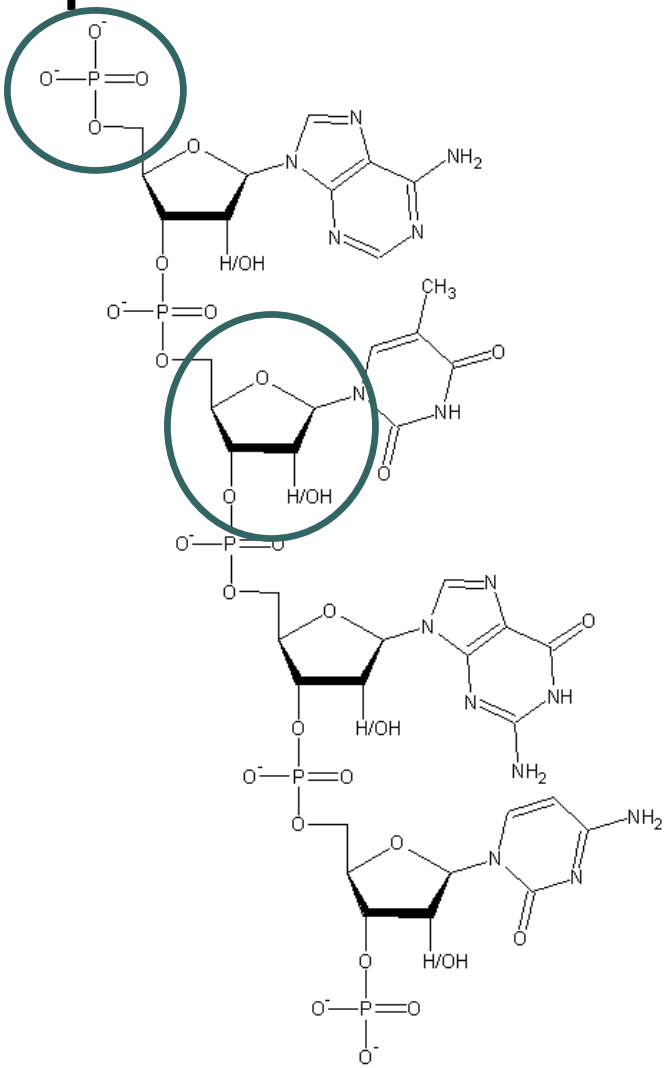


Dielectric response of proteins

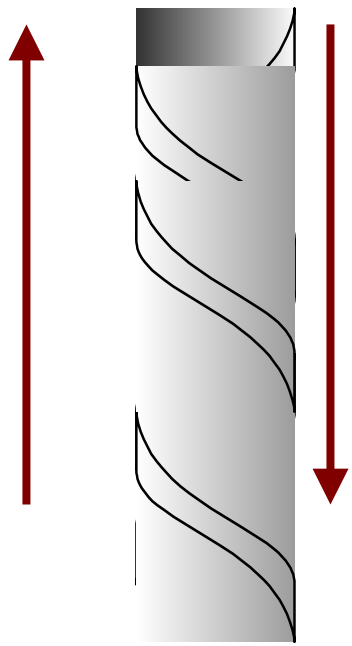
- Low net dipole moment
- ε'_r ~ 2-20
- β-dispersion
  - Broad orientational transition below 100 MHz
  - Frequency is inversely proportional to molecular volume



Nucleic acids (RNA, DNA)

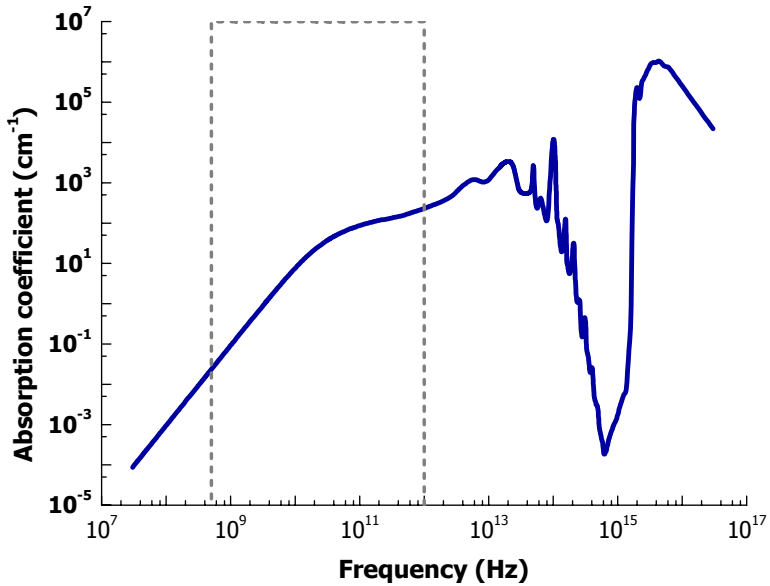


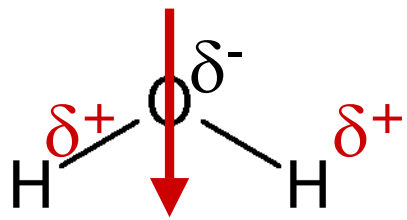
- Sugar, phosphate groups in single-stranded
- No net dipole moment when double-stranded



Dielectric dispersion of water

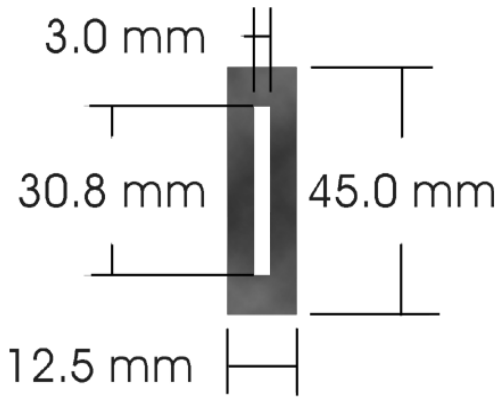
- Bulk water undergoes wide dispersion centered at 19.2 GHz
- Water bound to macromolecule undergoes dispersion at lower frequency
- Bound water can be used as reporter for macromolecular conformational change



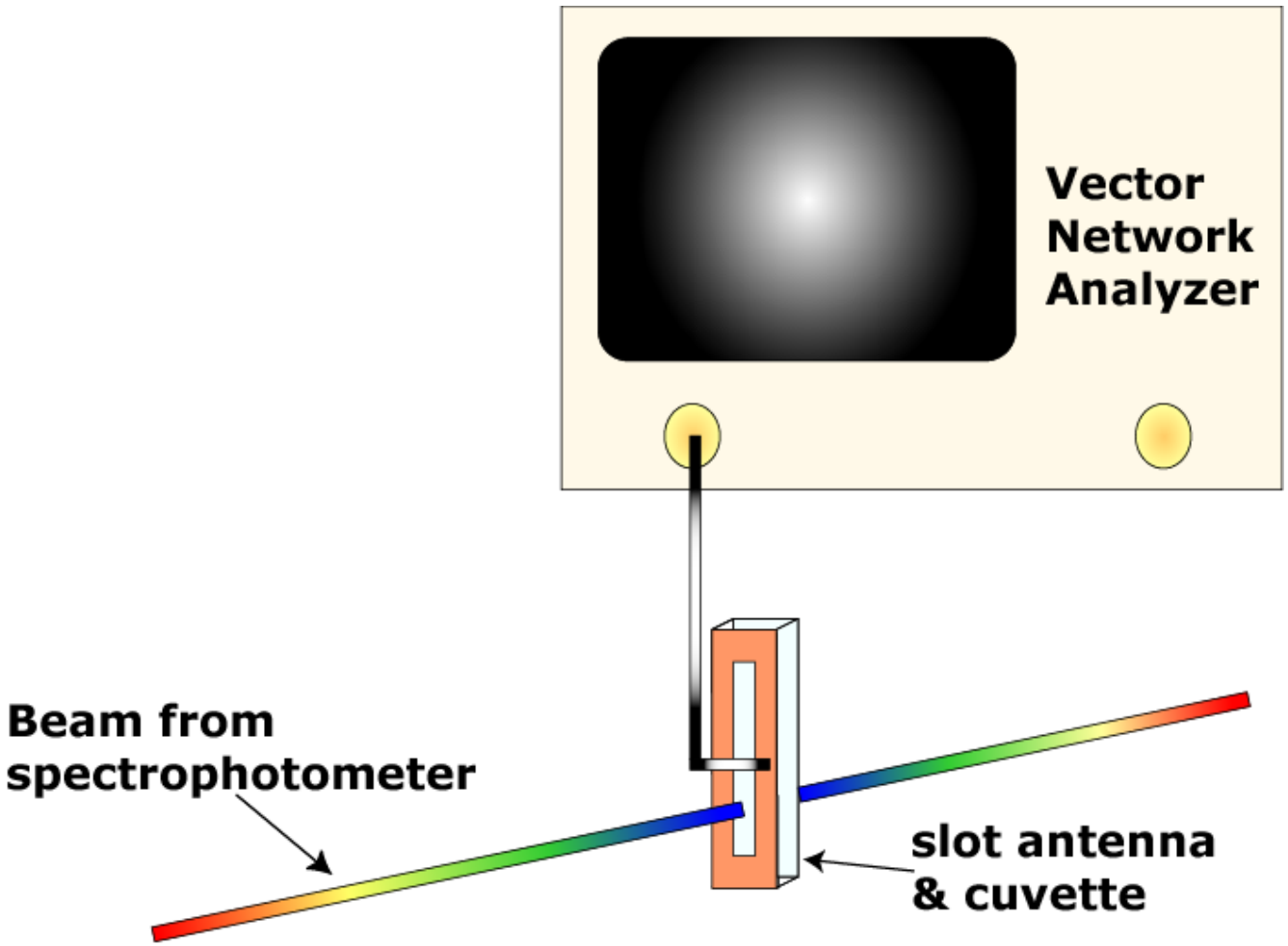


Resonant antennas, such as slots, enable microwave detection

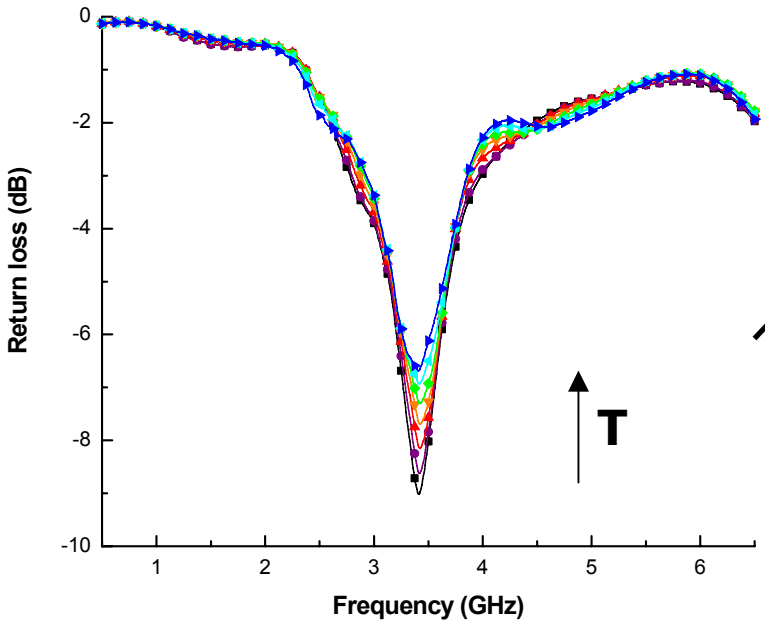
- Common antenna type in rf/microwave regime
- Slot length approx. equal to $\lambda_{resonant}$
- Fed by coaxial cable
- Attached to fused quartz cuvette to allow dual dielectric and UV/VIS measurements

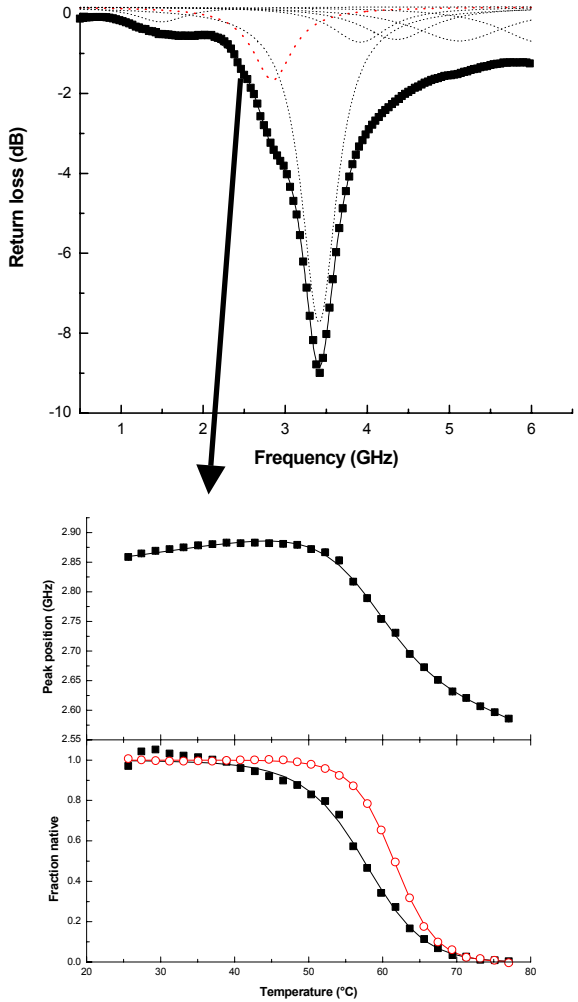


Experimental setup enables simultaneous microwave and optical detection



Dielectric response vs. temperature enables Tm extraction



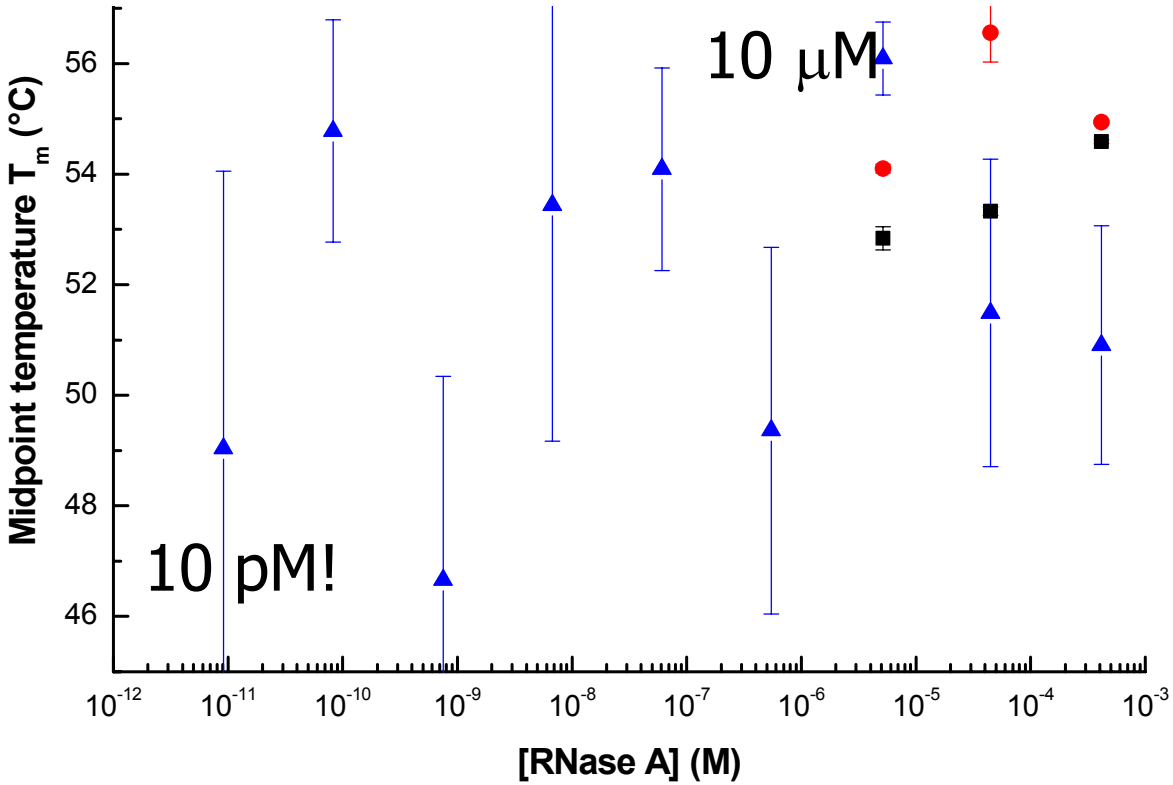


Protein unfolding/refolding studies

- Test system: unfolding/refolding of bovine pancreatic ribonuclease (RNase A)
- Three series of experiments:
  - Concentration series (19 pM - 680 μM)
  - pH series (pH 2.5 – 5.0)
  - Power series (-35 to 5 dBm; 18 μW to 1.8 mW)

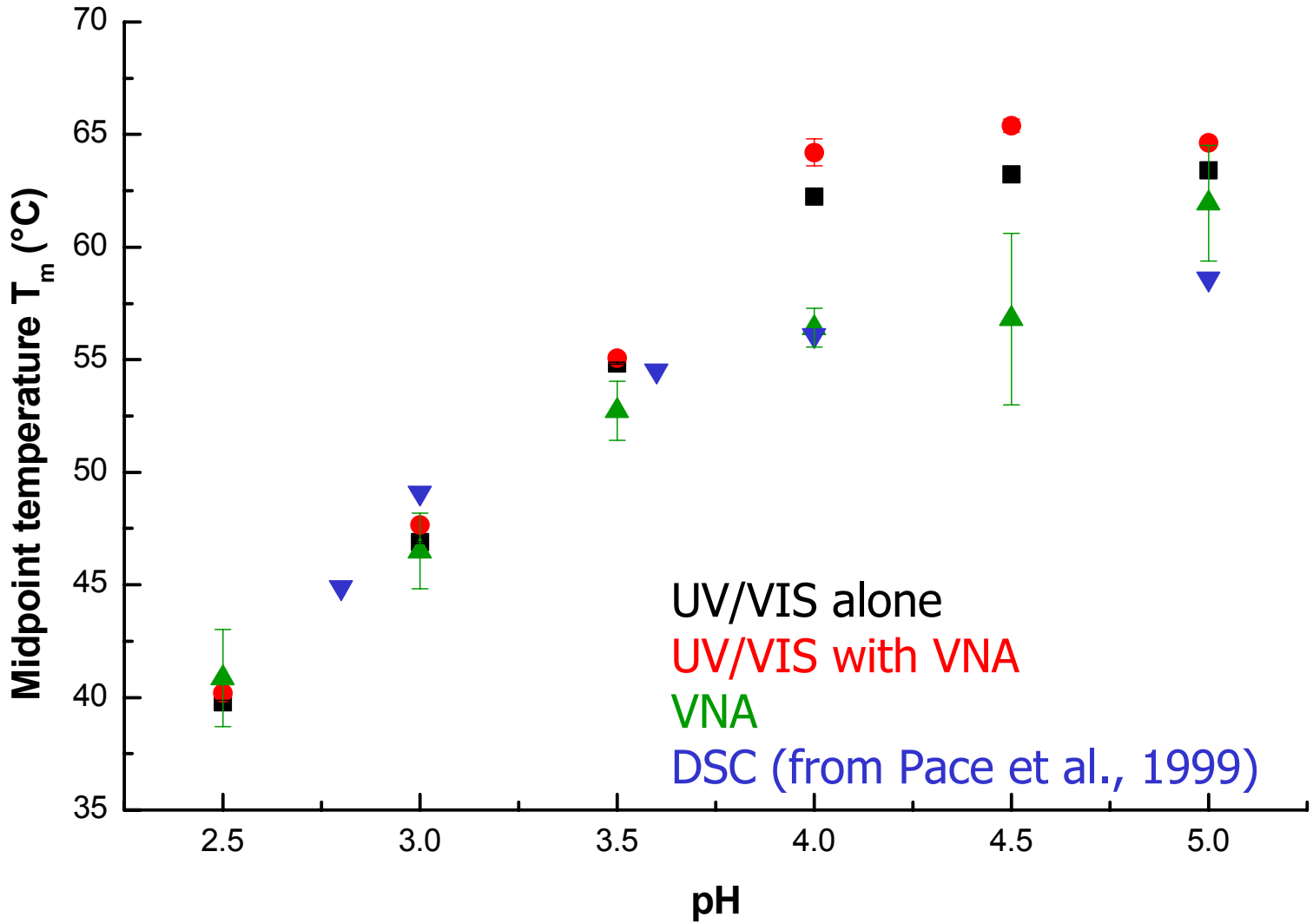
Concentration series shows ultrasensitive pM detection

- Little variation in midpoint temperature (T_m) with concentration
- T_m from UV/VIS equal with error with and without microwave power
- T_m from VNA measurements is lower and more noisy because of fixture variations



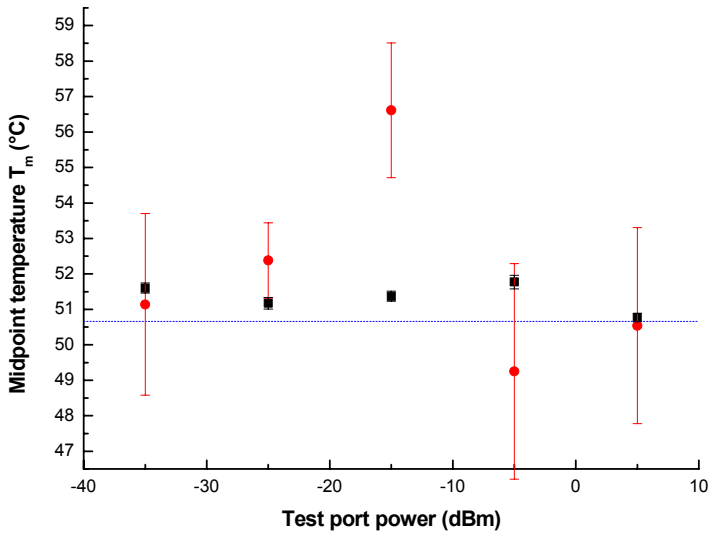
	Average T_m (°C)
UV/VIS alone	53.59 ± 1.00
UV/VIS with VNA	54.60 ± 1.76
VNA	51.76 ± 3.08

pH series results compare well with literature results



Power series shows no effect of microwaves on protein

- No evidence of increasing stabilization at low power
- T_m measured by UV/VIS in presence of microwave power slightly higher than T_m from UV/VIS alone



	Av. T_m (°C)
UV/VIS alone	50.66 ± 0.50
UV/VIS with VNA	51.23 ± 0.37
VNA	51.98 ± 2.82

Summary of protein unfolding results

- Results from VNA measurements parallel those from UV/VIS absorbance
  - Similar midpoint temperature (usually 1-3 °C lower)
  - Similar response to pH
  - No evidence of protein destabilization at low concentration
    - Unfolding/refolding curves measured to 19 pM
  - No evidence of protein destabilization at low power

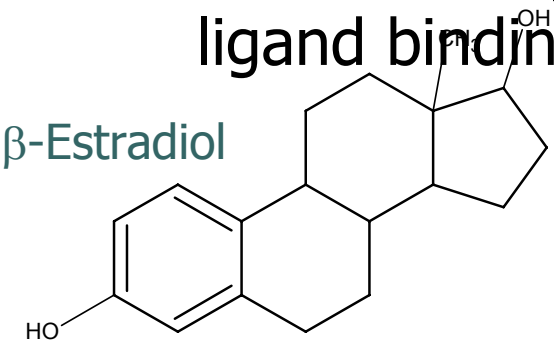
Methods: Ligand binding

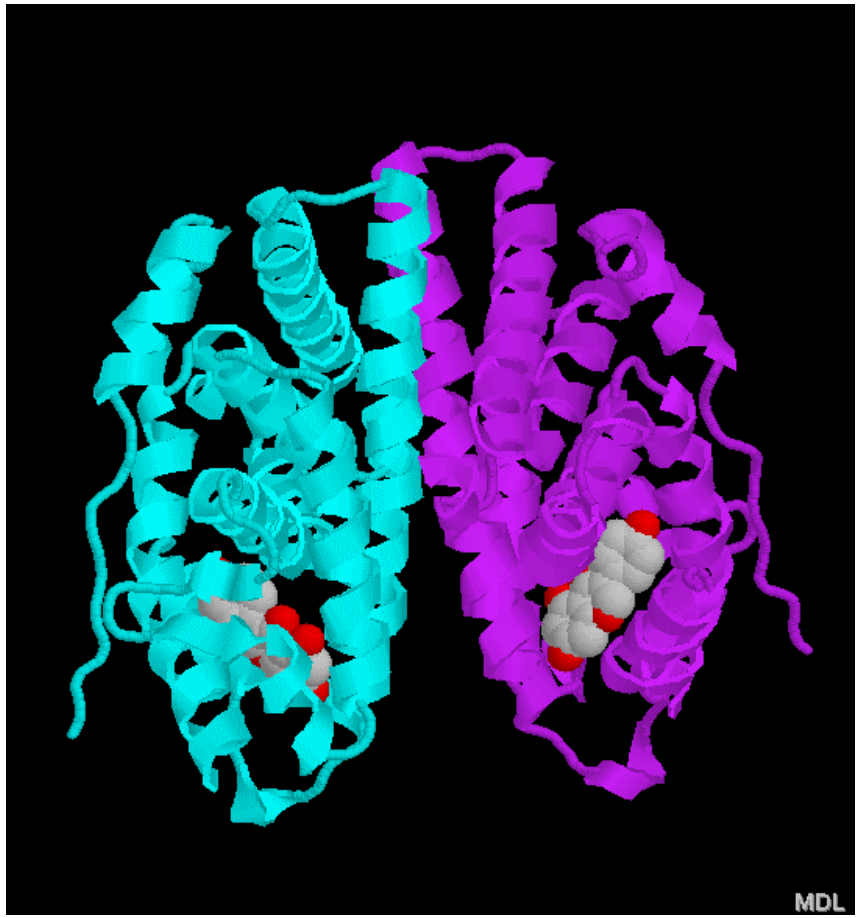
- Conventional methods require labelling or specialized equipment
  - Radio-labelling
  - Fluorescence or absorbance
  - Surface plasmon resonance
  - Isothermal titration calorimetry
- Idea: use slot antenna to deliver microwave power in range 10-20 GHz

Estrogen receptor β

- Target tissues:
  - Male and female reproductive systems
  - Heart
  - Bone
- Binds DNA upon ligand binding

17β-Estradiol

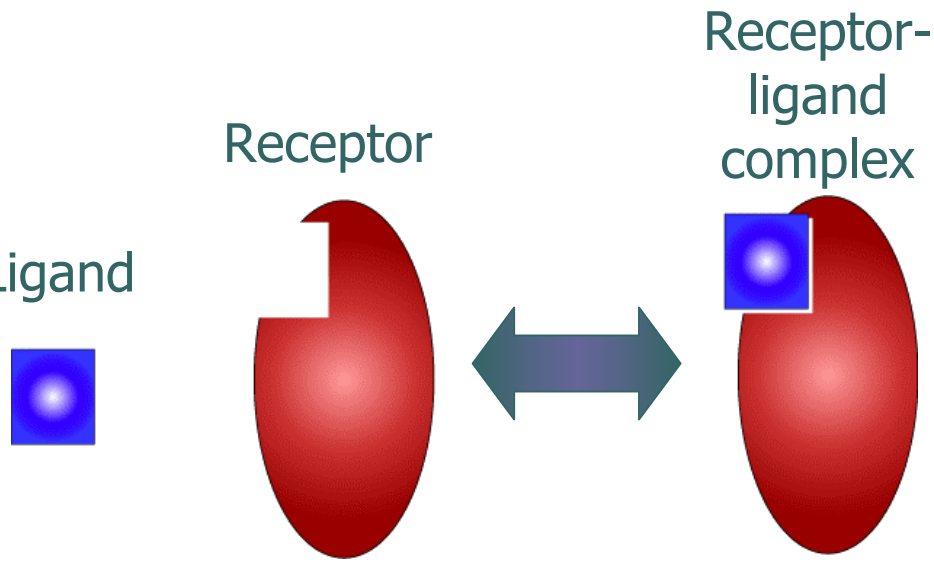




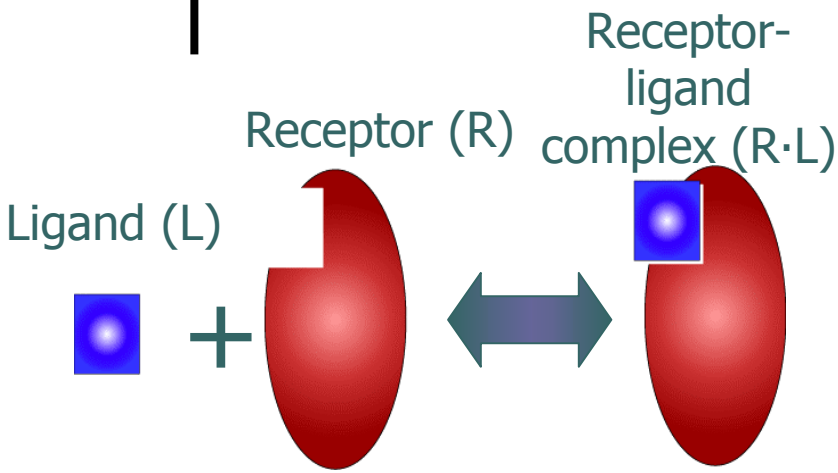
PDB code 1qkm

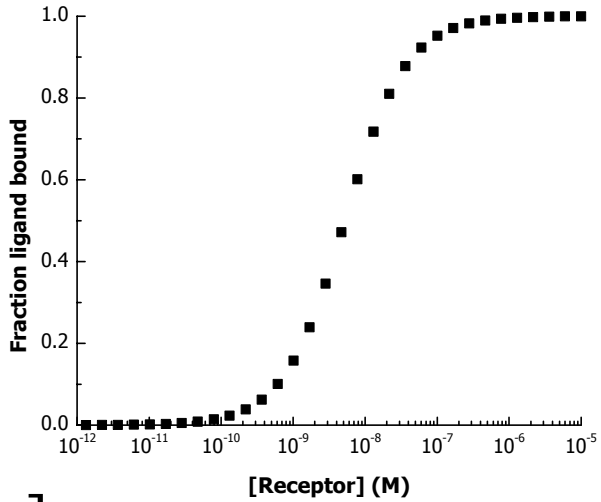
Fluorescence polarization

- Beacon: exploit tumbling rate of small ligand
  - Unbound: ligand tumbles quickly
  - Bound: ligand tumbles slowly
- Ligand: fluormone (fluorescein-labelled estradiol)



Single-Site Binding Model





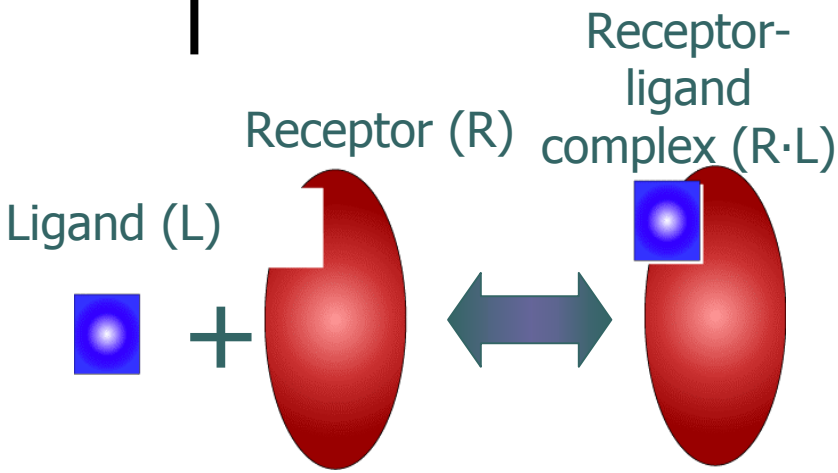
$$B \equiv [R \cdot L]$$

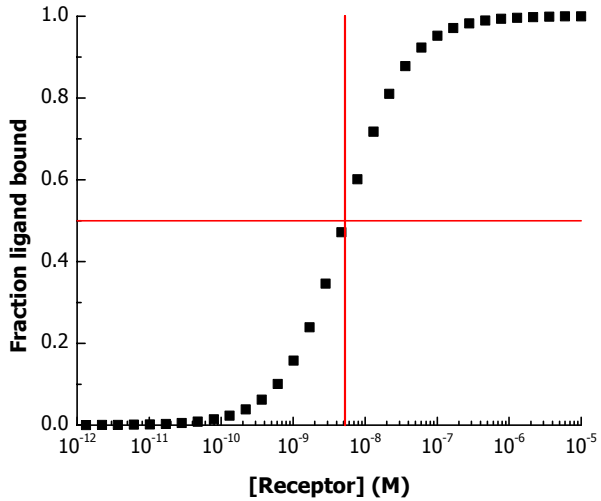
$$[L_{tot}] = [L_{free}] + B, [R_{tot}] = [R_{free}] + B$$

$$K_d = \frac{[L_{free}][R_{free}]}{[R \cdot L]} = \frac{([L_{tot}] - B)([R_{tot}] - B)}{B}$$

$$F_{bound} = \frac{B}{[L_{tot}]}$$

Single-Site Binding Model





When 50% of ligand is bound:

$$B = [L_{free}] = [L_{tot}]/2 = [R \cdot L]$$

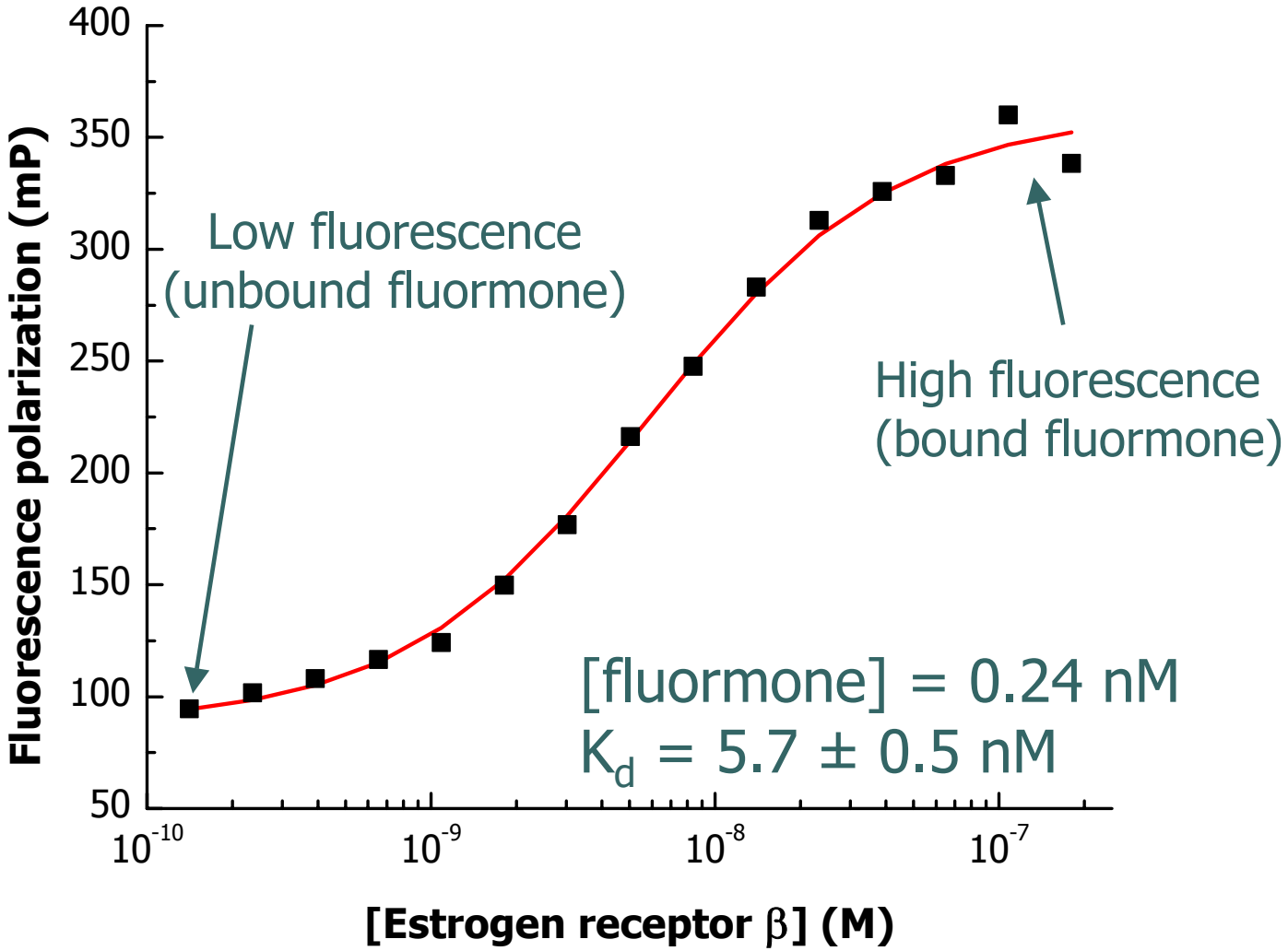
$$K_{1/2} = \frac{[L_{free}][R_{free}]}{[R \cdot L]} = [R_{tot}]_{50\%\text{binding}} - [L_{tot}]/2$$

$[R_{tot}]_{50\%}$ =5.25 nM

$[L_{tot}]$ = 0.5 nM

K_d = 5 nM

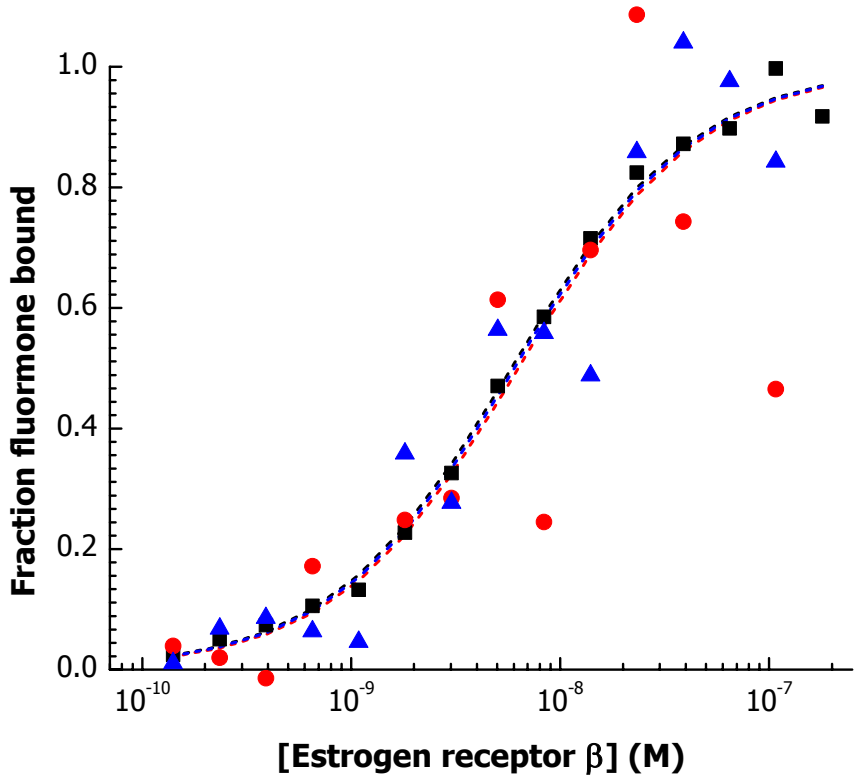
FP: ER-β/Fluormone binding



Combined setup can be reduced to chip scale

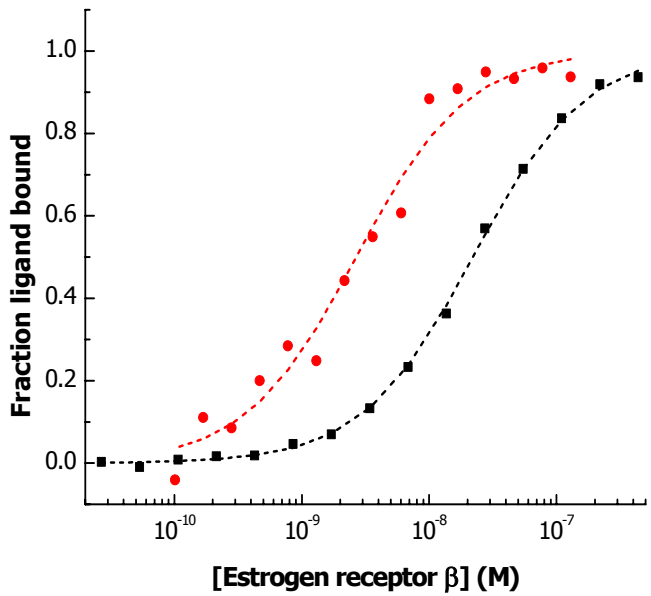
Fluormone binding results correlate with optical results

Source	K_d (nM)
Beacon	5.7
Peak 5 (11.75 GHz)	6.2
Peak 26 (18.66 GHz)	5.9



Estradiol vs. fluormone binding show effects of fluorescent label

Ligand	K_d (nM)	RBA
Estradiol	2.2	1
Fluormone	21.6	9.8



RBA = relative binding affinity

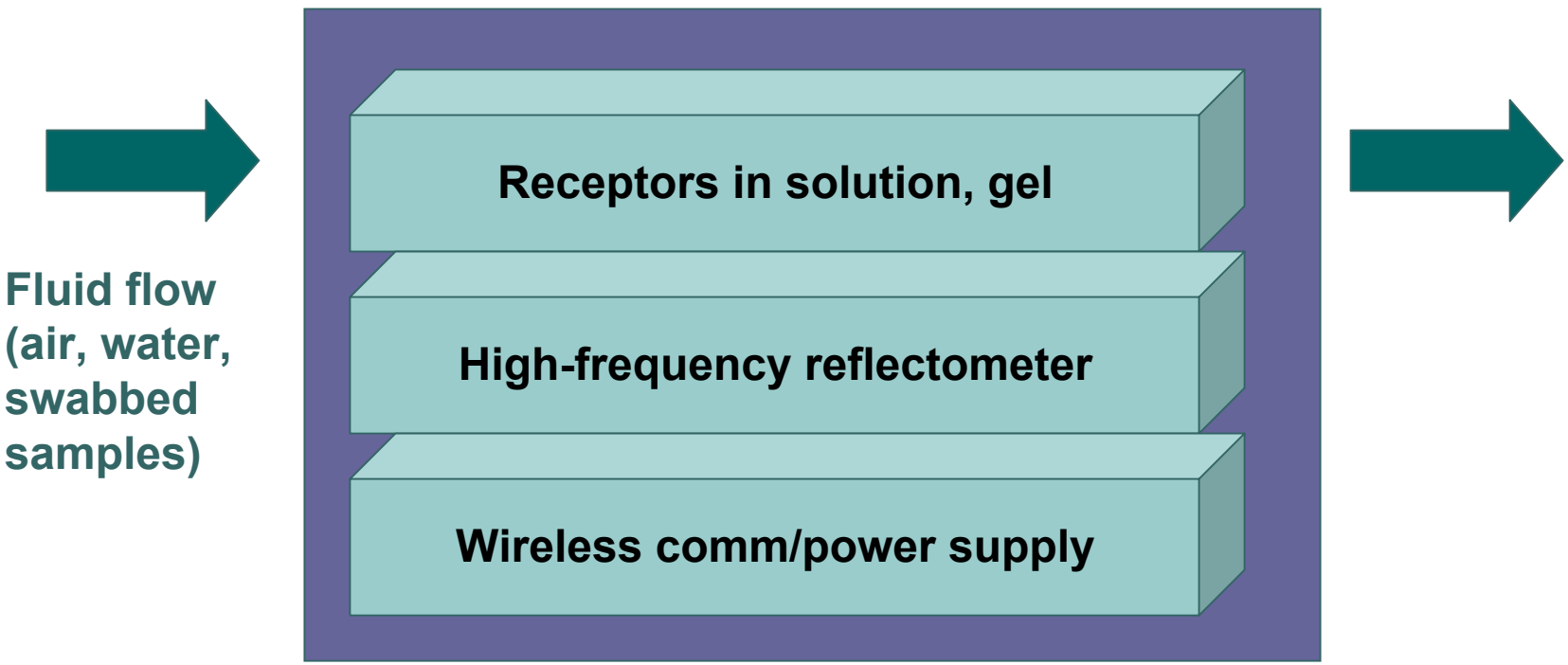
Summary: Ligand binding

- Slot antenna system can be used to detect ligand binding
  - Results from VNA compare well to results from Beacon
  - Binding of unlabelled ligands can be detected
  - Microwave power does not perturb the binding

Conclusions

- Slot antenna system can be used for simultaneous dielectric and optical observations of biological macromolecules
  - Unfolding/refolding of small globular protein
  - Receptor-ligand binding
  - Sensitive to very low concentrations
  - Unfolding or binding is not affected by microwave power under the conditions used

Field-deployable ultrasensitive biodetection is now possible in chip format



Acknowledgements

- Antenna fabrication: Alan Bettermann, Steve Limbach, Luke Palmer, John Peck (van der Weide laboratory)
- Equipment and reagents: PanVera Corporation (Madison, WI)
- Support:
  - Army Research Office
  - Office of Naval Research
  - NIH Molecular Biophysics Training Grant